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A New Trick for Cyclin-Cdk: Activation of STAT

New work in *Drosophila* demonstrates that *cdk4* loss causes phenotypes similar to the loss of JAK/STAT pathway components. Cdk4 overexpression can bypass requirements for JAK but not STAT. These results demonstrate a new function for Cdk4 and a new mode of STAT activation.

The STAT (*s*ignal *t*ransducer and *a*ctivator of *t*ranscription) family of transcription factors is essential for normal embryonic development and is thought to contribute to tumorigenesis. At the cellular level, STATs have been demonstrated to regulate proliferation, fate specification, and migration (Levy and Darnell, 2002). The most common mode of activation of STATs is via tyrosine phosphorylation by members of the *Ja*nus *k*inase (JAK) family, in response to cytokine signaling. Downstream targets of STAT that might mediate its proliferative effects include c-*myc*, cyclins, and raf (Bromberg, 2001; Kwon et al., 2000). However, the proteins that function together with STAT to regulate embryonic cell fate have been less well characterized. The paper by Chen et al. in this issue of *Developmental Cell* demonstrates a new function for cyclin-dependent kinase 4 (Cdk4) in the regulation of embryonic cell fate and segmentation in *Drosophila* by activating STAT, independent of JAK (Chen et al., 2003).

To identify additional genes that function with STAT to regulate cell fate and pattern formation, Chen et al. carried out a genetic screen for mutations that cause embryonic defects similar to those caused by mutations in the *Drosophila* JAK (called *hopscotch* or *hop*) or in the fly STAT (also known as *stat92E* or *marelle*). One locus identified in this screen was, surprisingly, *cdk4*. The similarities between the *cdk4* phenotype and those of *hop* or *stat92E* are striking. Embryos that lack both maternal and zygotic gene activity for any one of these loci show very similar cuticle patterning defects. In addition, embryos lacking *cdk4* show the same loss of expression of particular pair-rule genes in specific locations, for example, *even-skipped* stripe 3, as do embryos lacking *hop* or *stat*. Furthermore, mutations in *cdk4* resemble mutations in *hop* or *stat92E* in their effects on tracheal development (Chen et al., 2002).

These *cdk4* mutant phenotypes, especially the effect on pair-rule gene expression, are unlikely to be due to defective cell cycle progression because control of pair-rule gene expression occurs during the very rapid, early nuclear divisions in the syncitial *Drosophila* embryo. These division cycles lack G1 and G2 phases and therefore one would not expect *cdk4*, which normally promotes progression from G1 to S phase, to be required. Moreover, no defects were observed in the BrdU labeling patterns of either *cdk4* (Meyer et al., 2002) or *stat92E* (Chen et al., 2002) mutants at these stages. Therefore, these studies demonstrate a new role for Cdk4 in regulating pair-rule gene expression and pattern formation, independent of effects on cell cycle.

The best understood function of Cdk4 is that it works in a complex with Cyclin D to phosphorylate Rb, resulting in activation of E2F, which in turn activates Cyclin E-Cdk2 complexes (Sherr and Roberts, 1999). So after finding the similarity between the *cdk4* mutant phenotypes and JAK/STAT phenotypes, Chen et al. investigated whether Cyclin D and/or Cyclin E play a role in the pathway.

The authors demonstrate a variety of genetic interactions between *hop* and *stat92E* mutations and overexpression or loss-of-function of Cyclin D, Cdk4, Cyclin E, and Cdk2. The most striking of these effects is the observation that overexpression of either Cdk4 or Cyclin E can rescue the segmentation phenotype of an embryo lacking *hop* function but cannot rescue *stat92E* mutant embryos. This result strongly suggests that Cyclin-Cdk complexes can activate STAT independently of JAK activity. Whereas kinases other than JAK, such as epidermal growth factor receptor, src, abelson, and mitogen-activated protein kinase, have previously been shown to be capable of activating STAT, regulation by Cyclin-Cdk signaling appears to represent a previously unsuspected mode of STAT activation (Bromberg, 2001; Decker and Kovarik, 2000).

How then, do Cyclins and/or Cdks activate STAT? The authors were not able to detect phosphorylation of STAT by Cdk4. However, they were able to detect coimmunoprecipitation of Cdk4 and STAT when these proteins were overexpressed either in tissue culture cells or in embryos. Perhaps surprisingly, Cdk2 also immunoprecipitates with STAT. Moreover, coexpression of either Cyclin D-Cdk4 or Cyclin E-Cdk2 complexes with STAT in S2 cells results in a pronounced increase in the level of STAT protein detected by Western blotting. The same result is observed following coexpression of STAT and HOP. The authors conclude that Cyclin-Cdk complexes increase the protein stability of STAT. Consistent with this interpretation, embryos lacking *cdk4* function show a dramatic reduction in STAT protein expression, and embryos overexpressing Cyclin D and Cdk4 show increased STAT protein, by immunohistochemical staining. Although an effect on STAT transcription cannot be ruled out entirely, the available data support a direct effect on the STAT protein.

So if Cyclin-Cdk complexes activate STAT to alter

cell fate, does STAT function in the cell cycle? It was previously shown that embryos lacking maternal and zygotic *cdk4* function show defects in the BrdU labeling pattern specifically in endoreplicating tissues such as the midgut at state 14 (Meyer et al., 2002). Chen et al. show a similar BrdU labeling pattern in embryos lacking maternal and zygotic STAT. So whereas neither Cdk4 nor STAT is necessary for cell cycle progression in all dividing cells of the *Drosophila* embryo, the two proteins appear to serve similar functions in regulating progression through endoreplication cycles.

Future Directions

This study clearly demonstrates a new function for Cdk4 in regulating cell fate and pattern formation through STAT, and this role is apparently independent of effects on the cell cycle. Moreover, the study suggests a new mechanism for activating STAT. Many questions remain for future study, such as whether STAT and Cyclin-Cdk complexes bind to each other at physiological protein concentrations in the embryo. Another question is what signals control Cyclin D-Cdk4 expression and activation in this context. STAT protein levels have been previously reported to be dramatically lower in *hop* mutant embryos (Chen et al., 2002). Because Cyclin D is a known target of STAT in mammalian cells (Bromberg, 2001), one possibility is that activation of STAT via JAK leads to elevated expression or activity of Cyclin D and/or Cdk4, and this feeds back by activation of Cyclin E-Cdk2 to stabilize and activate STAT. However, further experiments are required to test this hypothesis. A third open question is whether the relationship between Cyclin-Cdk and STAT reported here is present in other cell types and organisms. Chen et al. investigated genetic interactions between these components in two other tissues, the developing eye and macrophage-like cells known as hemocytes. Although the relationship between JAK/STAT and Cyclin-Cdk in these tissues is not as straightforward to interpret, Chen et al. point out similarities between the effects on cell growth in these tissues and the mouse knockout phenotypes for Cyclin-Cdk and STAT. Finally, both of these pathways have been implicated in cancer, and it will be of interest to investigate whether crosstalk between Cyclin-Cdk and JAK/STAT contributes to tumorigenesis (Bromberg, 2001; Sherr and Roberts, 1999).

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A Structural View of Integrin Activation and Signaling

Integrins connect the matrix to the cytoskeleton and propagate structural order between the two systems. A series of elegant structural papers now provides a compelling explanation of how integrins perform this basic function.

The integrins are a class of adhesion receptors that link the extracellular matrix to the cytoskeleton and cooperate with growth factor receptors to promote cell survival, cell cycle progression, and cell migration (Giancotti and Ruoslahti, 1999). The integrins consist of an α and a β subunit. Each subunit has a large extracellular portion, a single transmembrane (TM) segment, and a short cytoplasmic domain (with the exception of β4). The N-terminal domains of the α and β subunits associate to form the integrin headpiece, which contains the ligand binding site, whereas the C-terminal segments traverse the plasma membrane and mediate interaction with the cytoskeleton and with signaling proteins. Integrins can signal in both directions: matrix binding promotes association of the integrin with the actin cytoskeleton and activates biochemical signals inside the cell (signaling; Giancotti and Ruoslahti, 1999). Conversely, intracellular signals can induce the integrin to bind to its matrix ligand (activation; Liddington and Ginsberg, 2002).

Recent structural studies buttressed by ingenious mutations and rotary shadowing EM analyses have revealed that integrin signaling and activation are mediated by large conformational changes that are propagated from the integrin headpiece to the cytoplasmic domains and vice versa, respectively (Liddington and Ginsberg, 2002; Shimaoka et al., 2002). The integrins exist in two major allosteric conformations, inactive (low-affinity state) and active (high-affinity state). The low-affinity state, which appears to be the default state, is maintained by a weak interaction between the C-terminal portions of the transmembrane segments of the two subunits (handshake or clasp). Upon binding to the integrin's headpiece, the matrix ligand induces conformational changes that are propagated along the integrin: the two legs, which are initially bent, undergo a "switch blade" movement, they straighten up, and the weak bond between the ends of the transmembrane segments of the α and β subunits is resolved. Release of this conformational restraint likely causes the α and β subunit cytoplasmic tails to move